This produced a far more definitive series of micrographs – one of submitochondrial particles with spheres, one with the spheres removed by Sephadex and urea, and a third, resembling the first, with the spheres reconstituted with F_1 (Racker & Horstman, 1967).

Racker's research localized the electron transport chain in the inner mitochondrial membrane and the ATPase in the spheres attached to the membrane (Figure 6.8c). This now presented a structural version of the problem biochemists had faced since the discovery of phosphorylation accompanying electron transport: How were the oxidation–reduction reactions of electron transport linked to ATP synthesis? The problem was now how to link activities localized in the inner membrane with activities in the attached spheres. For the most part, biochemists were still seeking chemical intermediates – the hypothetical compound C that, in Slater's scheme, formed an initial high-energy bond with the substrate and then transferred that bond to ADP or, in Lehninger's version (Figure 6.8), the postulated enzymes X, Y, Z that played that role at the three phosphorylation sites along the electron transport chain.

Radical Reconceptualization of Oxidative Metabolism

In 1961 Peter Mitchell, a maverick biochemist whose background had familiarized him with the enzyme-catalyzed translocation of chemical groups across membranes, advanced a revolutionary reconceptualization of oxidative phosphorylation. He suggested that the crucial intermediary was not chemical in nature but rather was a proton gradient across the inner mitochondrial membrane. The enzymes of the electron transport chain were so organized in the membrane that as the respiratory substrates were oxidized, protons (H^+) were discharged on one side of the membrane (the intermembrane space of the cristae) and OH^- ions were discharged on the other side (the mitochondrial matrix). Because the mitochondrial membrane is impermeable to H^+ and OH^- ions, a proton gradient bearing an electrical potential develops. When ATPase operates normally (that is, to hydrolyze ATP to ADP and inorganic phosphate), it also pumps ions across the membrane into the intermembrane space. But once a gradient has developed with significantly higher concentrations of protons in the intermembrane space than in the matrix, the ATPase can no longer can pump ions out of the matrix. This energy built up in the proton gradient provides the energy to drive the ATPase in reverse – synthesizing ATP from ADP and inorganic phosphate, rather than breaking it down (Mitchell, 1961; Mitchell, 1966). Because Mitchell's hypothesis, as shown schematically in Figure 6.8, made transport across a membrane